MINERAL SOLUBILIZING MICROBES ASSOCIATED WITH DIFFERENT CROPS AND THEIR FUNCTIONAL ROLES FOR PLANT GROWTH AND CROP PRODUCTIVITY

SYNOPSIS

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By

Rubee Devi (BS19PSBT002)

Department of Genetics, Plant Breeding and Biotechnology
Dr. Khem Singh Akal College of Agriculture,
Eternal University, Baru Sahib, Sirmour, Himachal Pradesh
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Name of the student	:	RUBEE DEVI
Registration No.	:	BS19PSBT002
Major Subject	:	Biotechnology
Name of Major Advisor	:	Dr. Ajar Nath Yadav (Assistant Professor, Senior Scale)
Name of Department	:	Department of Genetics, Plant Breeding and Biotechnology
Name of college	:	Dr. Khem Singh Gill Akal College of Agriculture

1. Title: MINERAL SOLUBILIZING MICROBES ASSOCIATED WITH DIFFERENT CROPS AND THEIR FUNCTIONAL ROLES FOR PLANT GROWTH AND CROP PRODUCTIVITY

2. Introduction

The increasing demand for food along with growing population has been a major concern across the world. The pressure to fulfill demand of the growing population and hunger problem has led to the advance of innovative scientific technologies and agriculture practices (Kour et al 2020d). Today, sustainability is a worldwide big deal as it helps you meet all your future agricultural needs without sacrificing soil quality. Traditional agriculture emerged from broadly three perspectives: a system of production to achieve food self-sufficiency, a concept of stewardship, and a way of sustaining rural communities. Plant-microbial biotechnology is an essential field that promotes human nutrition, food security, environmental protection and animal and plant conservation, eventually leading to holistic fundamental research in the sector of agriculture (Rastegari et al 2020; Yadav et al 2020a). Over the next few decades, the main focus of achieving sustainability will be on beneficial microbes. Microbes are expected to pave the way for the green revolution in agriculture, and they will undoubtedly be a great alternative to sustainable organic farming approaches (Yadav et al 2020a; Yadav et al 2020b). The main purpose of organic forming is to grow abundant and healthy crops can be produced without using chemical inputs (fertilizers and pesticides) for crop production and protection. Pesticides pollute ecosystems; reduce microbial diversity in the natural environment making plants more vulnerable to pests and diseases. The use of bio-based fertilizers and pesticides for agro-environmental sustainability is a promising strategy to boost crop productivity and manage plant diseases (Kumar et al 2021; Singh et al 2020).

Microorganisms are the greatest varied and abundant group of organisms on the Earth (Fierer and Jackson 2006). Microbes are reported worldwide as plant microbes and soil microbes (Yadav 2021b and 2021c). Plant-associated microbes (endophytic, epiphytic and rhizospheric) play an vital role in stimulating plant growth,development like nutrient cycling and crop productivity and sustaining the fertility of soil and also useful in the developing strategies to facilitate growth of plant under normal and

stressful (biotic and abiotic) condition (Yadav et al 2020a; Kumar et al 2021b; Rana et al 2020a). Plant and rhizosphere soils are natural valuable resources that harbor diverse microbial species, and these microbes play significant role in the ecosystem functioning (Verma et al 2017; Yadav et al 2021). Microorganisms employ diverse mechanisms to improve plant growth directly or indirectly, including fixation of nitrogen, solubilization of nutrients like phosphorus, potassium, zinc, and selenium by producing organic compounds (Kaur et al 2021; Suyal et al 2021; Yadav 2021a). Other PGP microbial mechanism are production of siderophores, ammonia, hydrogen cyanide (HCN) and secondary metabolites that helps in alleviation of stress mediated by plant pathogens (Gupta et al 2015). Many PGP microbial strains such as *Azotobacter*, *Azospirillum*, *Agrobacterium*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Paenibacillus*, *Pseudomonas*, *Serratia* and *Streptomyces* are commercially used as PGP microbes for plant growth and development.

Minerals solubilizing microbes could use for stimulating growth and productivity of plant crop. Macro (N, P, K, Ca, Mg, S, C, O and H) and micronutrients (Fe, B, Cl, Mn, Zn, Cu, Mo and Ni) are needed for well-functioning of plants. Phosphorus (P) and potassium (K) are the two most important and widely distributed macronutrients, both of which are found in insoluble form in agricultural soils. Mineral-soluble microbes have capability to soluble various macronutrients, easily accessible to plant that help plant growth, development and enhance fertility of soil. Lack of these nutrients may lead to growth impairment, yield depression and altered plant metabolism. These problems may be solved using MSMs. Mineral solubilizing microorganisms could be used as a bioinoculants to promote plant growth and provide more balanced nutrition to many crops, as well as to improve the stability and stability of ecosystems.

Looking the importance of mineral solubilizing microbes in agriculture, the present work would be carried out with following objectives

1. Isolation and characterization of microbes associated with crops growing in hilly regions
2. Screening of isolated microbes for mineral solubilizing attributes
3. Molecular characterization of selected mineral solubilizing microbes
4. Development of microbial consortium of mineral solubilizing microbes for plant growth and enhanced crop production
5. Evaluation and validation of mineral solubilizing microbes for plant growth promotion under controlled and natural conditions

The rationale of the proposed study

Minerals are most essential elements for growth of plant and development. These inorganic nutrients occur naturally in the soil and are important for the functioning of animals and biological growth

and development of plants. Minerals are divided into two categories such as macronutrients (N, P, K, Ca, Mg and S), and micronutrients (Zn, Se, Fe, Mn, Al, Ni, B, Cu and Mb), micronutrients are required by the plant in very few amounts, whereas macronutrients are needed in large quantities.It is essential for acidity and buffer action, catalytic effects, osmotic potential of cells, influence on permeability of cytoplasmic membranes, toxic effects, balancing effects, antagonistic effects, and electrostatic neutrality maintenance and phloem movement. It is also involved in the absorption of minerals from soil, and transport within plants and other function like metabolic activities, nucleic acid and synthesis of protein. Minerals are present in a soluble form in the soil, which is then dissolved by microorganisms and made available for uptake by plants. In soluble form of mineral are available in soil that is solubilized by microbes and finally available to plant for uptake. MSMs will not only provide an alternative and application of less chemical fertilizers, but also improve plant health and soil fertility as an ecofriendly approaches. There are very few reports on MSMs and their applications in agriculture.

3. Expected new knowledge

- Advancement in taxonomy of microbes isolated from cereal crops growing in hilly regions
- Identification of novel species/strain of mineral solubilizing microbes
- Development of mineral solubilizing bio- inoculants for cereal crops growing in hilly regions

4. Review of literature

Chemical pesticides and fertilizers are used in traditional agriculture to enhance the crops quality and the yield. Frequent and excessive use of these chemicals has adverse environmental impacts, disturbing the ecological balance of the soil and eventually making plants more vulnerable to pests and diseases. Therefore plant growth promoting (PGP) microorganisms can be a complementary way to reduce the use of chemical fertilizers in the agriculture. Microbes are the key determinants of plant growth and productivity. Mineral solubilizing microbes are soil and plant microbiome and have capability to solubilize the diverse minerals (P, K, Zn and Se) by using different mechanism. Mineral soluble microorganism (MSMS) can play a vital role in supplying minerals to plants ecosystem. Microorganisms are living organisms that are found across of planet earth that are so small to be seen with the naked eye. Microbes are categorized into four major groups namely archaea, algae, bacteria, fungi, and protozoa.

Rhizosphere (zone around the root) is basically the narrow zone surrounded and influenced by root of plant and is a hot spot for many organisms. The different groups of microbes which have been reported from rhizospheric microbiomes include phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. Many studied have been done on soil microbes essential for increases production of crop and health including *Acinetobacter*, *Bacillus*, *Enterobacter*, *Micrococcus*, and *Pseudomonas* isolated from

root associated soil of French bean (Kumar et al 2012). In a study, *Acinetobacter* sp., *Bacillus subtilis*, *Duganella violaceusniger, Paenibacillus durus, Pseudomonas thivervalensis*, *P. lini*, *Pantoea ananatis*, *Serratia marcescens* and *Stenotrophomonas* sp. these microbes were isolated from soil associated with the roots of wheat (Verma et al 2014), *Bacillus*, *Stenotrophomonas* and *Acetobacter pasteurianus* from rhizospheric soil of wheat (Majeed et al 2015), *Pseudomonas stutzeri, Bacillus subtilis*, *Stenotrophomonas maltophilia*, and *Bacillus amyloliquefaciens* isolated from rhizospheric soil of cucumber (Islam et al 2016), *Pseudomonas aeruginosa*, *P. fluorescens* and *Bacillus subtilis* from rhizospheric soil of maize (Karnwal 2017), *Pseudomonas* sp., *P. palleroniana, P. proteolytica*, and *P. azotoformans* isolated from crop growing Himalayan regions (Adhikari et al 2021) and *Azospirillum brasilense* and *Enterobacter* sp., from rhizospheric soil of sorghum (da Silva et al 2018), *Bacillus pumilus*, *B. subtilis*, *B. licheniformis*, *B. safensis* and *B. cereus* isolated from rhizospheric soil of chickpea (Sharma et al 2019). Another reported species *Bacillus cereus* isolated from rhizosphere (Saqib et al 2020). Two plant growth-promoting bacteria *Bacillus subtilis* and *B. megaterium* isolated from sugarcane rhizosphere (Chandra et al 2021).

One of the most significant taxonomic groups of fungi is eukaryotic living organisms, found throughout the planet. One of the most imperative taxonomic groups of fungi is eukaryotic and heterotrophic living organisms, found throughout the planet. These PGP fungi include the genera *Aspergillus, Fusarium, Penicillium, Piriformospora, Phoma, Rhizoctonia and Trichoderma,* which have the capability to stimulate various growth-related traits of plants (Hossain et al 2014). Several fungi have been reported including, *Rhizoctonia solani Fusarium* spp., *Sclerotium rolfsii*, *Verticillium dahliae* and *Sporisorium reilianum* from diverse host plants (Han et al 2016). Three species of arbuscular mycorrhiza fungi *Claroideoglomus etunicatum and Funneliformis mosseae* were isolated from teak, rhizosphere soil, while *Gigaspora* sp. was sorted out from the *Asparagus* rhizospheric soil (Chaiyasen et al 2017). *Lecanicillium psalliotae* (Kumar et al 2018), *Trichoderma* spp. and *Piriformospora indica* sorted out from the soil collected from Northern Iran (Mazandaran Province) (Yaghoubian et al 2019), *Torulaspora indica*, *Wickerhamomyces anomalus* (Limtong et al 2020).

Archaea are unique microorganisms that exist in extreme ecological regions of high temperature, pH and salt. Archaea can exist independently or attached to the plant's rhizosphere. Archaea have been reported as ubiquitous nature and present in an inclusive range of environments from hypersaline habitats. A few studies have been reported on archaea including *Halolamina*, *Halococcus*, *Natrinema*, *Halosarcina*, *Haloterrigena*, *Haloferax*, *Haloarcula*, *Natrialba*, and *Halobacterium*, were isolated from different plants associated soil growing in Rann of Kutch, Gujarat, India (Yadav et al 2015), *Crenarchaeota, Euarchaea* from *jatropha curcas* (Dubey et al 2016), In another investigation, archaea were reported *Haladaptatus paucihalophilus, Haloarcula argentinensis, Natrinema altunense, N.*

pallidum and *Natronoarchaeum mannanilyticum* isolated from surface of Rann of Kutch (Yadav et al 2019).

Plant microbes have capability of colonizing the endophytic, rhizospheric and phyllospheric. The endophytic microbe colonizes the inside of plants parts, i.e. leaves, root, stem, flower, fruit or seeds, without exerting any deleterious effect on host plants. These microbes were isolated from a different variety of crops/plants including *Agrobacterium*, *Acidovorax*, *Burkholderis*, *Methylobacterium*, *Rhodopseudomonas* and *Ralstonia* from rice (Piromyou et al 2015). A huge number of endophytic bacteria belonging to different species of genera including *Acinetobacter calcoaceticus, Bacillus* sp., *Enterobacter cloacae*, *Ochrobactrum haematophilum*, and *Pseudomonas sojae,* have been sorted out from soybean (Zhao et al 2018). In a study Borah et al (2019), reported *Bacillus* sp., *Brevibacterium sediminis*, *Lysinibacillus xylanilyticus* and *Paenibacillus* sp. from tea plants. *Acinetobacter guillouiae* isolated from maize (Rana et al 2020b) and *Pseudomonas brenneri*, *Ewingella americana*, and *Pantoea agglomerans* were isolated from maize (Rana et al 2021).

Phyllospheric (colonize the aerial parts), microbes colonize the microbes resides on the above-ground section of the different part of the plants including flowers, fruits, leaves, and stems. Several microbes like *Pseudomonas* from flower samples of apple trees (Steven et al 2018), *Candida blattae*, *C. humilis* and *Saccharomyces cerevisiae* from bark of *Ficus sycomorus, Candida glabrata* from bark of *Ficus vasta*, *Rhodotorula mucilaginosa* from mango fruits, *Yarrowia lipolytica* from sugarcane, *Kodamaea ohmeri* from guava fruits (Dabassa Koricha et al 2019) have been isolated and characterized. Similarly (Abadi et al 2020) reported *Bacillus*, *Enterobacter*, *Kocuria, Pseudomonas*, *Microbacterium*, *Stenotrophomonas* and *Pseudarthrobacter* isolated from maize phyllosphere.

Microbes are important in acquiring of nutrients for plant growth and crop production. Different mechanism of PGP including MSMs the mineral phosphorus forms are represented by primary minerals in soil including apatite, hydroxyapatite, and oxyapatite. Phosphorus is found two chemical form like organic P (Po) and inorganic P (Pi) in soil. In soil, the inorganic form of phosphorus is present in about 35– 75% of the total P pool. Inorganic Pi is present as a mineral source like Ca, Fe and Al phosphate in the soil. In which calcium phosphate mainly exist in the form of apatite like hydroxyapatite ($Ca_5(PO_4)_3OH$), fluoroapatite ($Ca_5(PO_4)_3F$) and francolites ($Ca_5(PO_4, CO_3)_3F$), that are the primary source of Pi in natural or alkaline soil, however in acidic soil Fe and Al exist in the form of phosphate as oxy(hydr)oxides such as strengite ($FePO_4.2H_2O$), variscite ($AlPO_4.2H_2O$) and wavellite ($Al_3(OH)_3(PO_4)_2 \cdot 5H_2O$). Another organic form (Po) is present about 30-60% in the soil. Inorganic forms of organic phosphate (Po) found in soil include inositol, phosphates, phospholipids and nucleic acids, as well as monophosphorylated carboxylic acids, organophosphorus (phytin), sugar phosphate and teichoic acid. In a study, Nath et al (2015) isolated *Penicillium sclerotiorum* and *Aspergillus niger* from soybean

were found to solubilize phosphorus *in vitro* as well as increased the growth of soybean. Similarly, Ogutand Er (2016) reported *Bacillus* sp. from rhizospheric soil of wheat were found to solubilize P, greatly increasing plant root growth and also encourages the uptake of nutrients other than P (e.g., K). Another study, Wang et al (2017) isolated *Bacillus cereus* from rhizosphere soils, solubilize phosphors and enhancing plant growth parameters of Chinese cabbage (*Brassica campestris* ssp. *chinensis*).Tahir et al (2018) examined the effect of bio-organic phosphate or in combination with phosphorus solubilizing bacteria *Bacillus* was isolated from rhizosphere of wheat, with enhancing growth and productivity of two wheat cultivars (Galaxy-2013 and Punjab-2011). Kour et al (2019) reported *Pseudomonas libanensis*, *Acinetobacter calcoaceticus* and *Penicillium* sp. from rhizospheric soil of wheat and foxtail millet (*Setaria italica* L.), to solubilize a significant amount of P under the rain-fed condition with increasing growth of plants (Kour et al 2020c). In addition, *Bacillus, Kluyvera, Buttiauxella, Meyerozyma and Penicillium,* isolated from rhizosphere soils at five bamboo forests in subtropical China, were solubilize of P *in vitro* from organic and inorganic P pools (Xing et al 2021).

Potassium (K) is a key plant nutrient that influences growth and development of plant. It's crucial for the synthesis of cells, cellulose, enzymes, proteins, starch and vitamins. K contributes in nutrient transport and uptake, thereby increasing the production of quality crops and providing resistance to plant diseases. K solubilizing microbes KSMs play a vigorous role in the solubilization of potassium from soil by the processes including acidolysis, capsule absorption, and complication through extracellular polysaccharides, lowering of pH, enzymolysis and organic acid production (Meena et al 2016). Chemical K fertilizers are the largest available sources of K in the soil; hence, vast amounts of K fertilizers can encourage promoting K availability for plant absorbed. KSMs are important for plant growth regulating substances, antibiotics production, and biodegradation of organic matter and also helpful in cycling of nutrients in the soil for crop productivity and environmental sustainability. KSMs including *Acidothiobacillicus ferrooxidans*, *Bacillus mucilaginosus, B. edaphicus, B. megaterium* and *Pseudomonas* spp., and have already been shown to release K from K- bearing minerals by producing organic acid. The mechanism of K-solubility may be mainly due to the production of organic acids that vigorously dissolve either rock potassium or chelate silicon ions to move K into solution (Kour et al 2020b). Saha et al (2016) reported *Bacillus licheniformis* and *Pseudomonas azotoformans* from rhizospheric soil, were able to solubilize potassium and also enhancing plant growth and crop productivity. Bagyalakshmi et al (2017) reported *Bacillus subtilis* isolated from rhizosphere soil, which was also able to solubilize potassium and promote plant growth. Similarly Khanghahi et al (2018) reported *Pantoea agglomerans*, *Rahnella aquatilis* and *Pseudomonas orientalis* from isolated from rhizospheric soil of paddy, it able to solubilize potassium along with increased the grain yield. Pramanik et al (2019), isolated *Bacillus pseudomycoides* from tea-growing soil and identified as significantly enhanced K availability in soil, and that in turn

facilitated K uptake by tea plants. Sun et al (2020), reported *Burkholderia* sp. from rhizosphere soil, it soluble of potassium and increasing agricultural yield.Another study, two bacterial strains *Bacillus licheniformis* and *Pseudomonas azotoformans* isolated from rhizospheric soil, it also able to solubilize potassium and they were increasing k efficiency in soil (Azizah et al 2021).

Plants need zinc to carry out their physiological and metabolic processes. Plant uptake zinc in a soluble form as a divalent cation (Zn^{2+}), but there is a lot of zinc available in soil in the form of insoluble zinc (Alloway 2008). To enrich the soil profile uses zinc solubilizing microbes to encourage the essential growth of plant and soil health and increasing crop yield (Kloepper et al 2004). Zinc solubilizing microorganisms solubilize zinc by several mechanisms such as acidification, lowering of pH and also produce organic acid in soil. Other mechanisms of zinc solubilization include the production of siderophores and proton exclusion, oxidative system on cell membrane and also chelated ligands. Many zinc soluble microbes have shown increased plant growth and zinc content that inoculation with plants. These microbes include *Azospirillum, Bacillus aryabhattai, Bacillus* sp., *Pseudomonas* and *Rhizobium*. Zinc solubilizing bacteria enhanced nutrition and zinc uptake in *Zea mays* L. *Bacillus* strains affecting growth, yield and zinc biofortification of soybean and wheat have also been reported through scientists by Khande et al (2017). Abaid-Ullah et al (2015), reported *Serratia grimesii, S. liquefaciens, S. marcescens* and *Bacillus thuringiensis*, isolated from wheat rhizosphere and improve grain yield and Zn content of wheat as compared to chemical Zn fertilizer. *Stenotrophomonas maltophilia, Mycobacterium brisbanense, Enterobacter aerogenes, Pseudomonas aeruginosa,* and *Xanthomonas retroflexus* have been isolated to solubilize insoluble forms of zinc into soluble form (Sunithakumari et al 2016).

In addition, Shaikh and Saraf (2017) reported *Trabusiella* sp., *Aeromonas* sp., *Arthrobacter* sp. and *Exiguobacterium aurantiacum* from the rhizospheric soil of agricultural field, these microbes have ability to soluble of zinc and enrichment of plant growth and nutritional quality of grain in soil. Two bacterial strains *Agrobacterium tumefaciens* and *Rhizobium* sp. from the rhizosphere of barley (*Hordeum vulgare* L. cv. Europa) and tomato (*Solanum lycopersicum* L. cv. Marmande) plants showed a solubilizing efficiency for ZnO (Khanghahi et al 2018). The effect of two P-Zn-solubilizing bacterial strains (*Bacillus* sp. strain AZ17 and *Pseudomonas* sp. strain AZ5) was evaluated on the growth of chickpea plants. In another investigation Zaheer et al (2019), reported *Pseudomonas* sp. zinc solubilizing bacteria from rhizospheric soil of chickpea. Similarly, *Paenibacillus polymyxa*, *Ochrobactrum intermedium*, *Bacillus cereus*, *Streptomyces*, *Stenotrophomonas maltophilia* and *Arthrobacter globiformi* were isolated from chickpea rhizosphere, these microbes able to solubilize zinc, making them valuable for improving Zn biofortification in chickpea (Batool et al 2021).

Among micronutrients, iron (Fe) plays an essential nutrient for proper growth of plant, human health and livestock (Roriz et al 2020). Biofortification is an important process of consciously producing

food enriched with requires micronutrients by a transgenic approaches, mineral biofertilizers and conventional breeding (Murgia et al 2012). The siderophore is a low molecular weight of iron compound with a high affinity for iron. These are categories into four different types like carboxylates, catecholate, hydroxamates and pyoverdines (Crowley 2006). It has well established that siderophore improves plant growth, development and increasing content of micronutrients. Siderophores production is one of the best important mechanisms of microbes for iron acquisition since iron is an essential mineral and needed for various metabolic processes such as antibiotics, biosynthesis of nucleic acids, cytochrome, electron transport chain, photosynthesis, porphyrins, pigments, oxidative phosphorylation, tricarboxylic acid cycle, toxins and vitamins. Many studies have shown that plants uptake iron via microbial siderophores and several microbial stains have been linked to production of siderophores, microbes, including *Bacillus aryabhattai* (Mumtaz et al 2017), *Curtobacterium*, *Plantibacter*, *Pseudomonas*, *Stenotrophomonas* (Costerousse et al 2018). Kour et al (2020a) reported *Streptomyces laurentii* and *Penicillium* sp. are known to produce siderophores and it can be used for the biofortification of iron. Sarwar et al (2020), reported siderophore producing bacteria species including *Bacillus subtilis*, *B. halotolerans*, and *B. safensis* isolated from rhizospheric soil of groundnut (*Arachis hypogaea* L.) and useful in reducing the iron deficiency in groundnut. Mushtaq et al (2021) reported *Acinetobacter calcoaceticus* and *Bacillus simplex* were isolated from the potato rhizosphere; these were able to producing siderophores and improvement in the plant physiological process.

Selenium (Se) is a metalloid, shows a number of beneficial and toxic effects on organisms which based on the amount of Se available in the soil for plant uptake, accumulation in foods, and its daily consume by humans and other organisms. Soils have low selenium accessibility, producing plant with low Se content. Currently, Se is being studied as an important micronutrient due to its antioxidant capacity and beneficial effects on human health. Inorganic forms include selenite (SeO_3^{2-})/selenate (SeO_4^{2-}) salts, whereas organic forms are Se products from the decay of Si-rich plant material. Various studied have available for Se biofortification with plant growth promoting and increasing Se content in plants. Microbes like *Bacillus*, *Paenibacillus*, *Klebsiella*, *Enterobacter*, and *Acinetobacter* isolated from wheat plants growing in the field, these microbes has ability to high Se tolerance, their promoting effects on plant growth, and their protection against *Gaeumannomyces graminis* (Durán et al 2014). In a study, Wang et al (2016) reported *Caulobacter vibrioides* was isolated from a selenium mining area in Enshi, southwest China that strongly solubilizes selenium (Se) mineral into Se (IV) and also produces the phytohormones IAA and promotes plant growth.

Abiotic and biotic stresses limited the plant growth and its productivity. Abiotic stresses are temperature Stresses, soil salinity, flooding, water deficient stress, mineral deficiency and heavy metal toxicity, whereas biotic stresses are those imposed by various phytopathogens including bacteria, fungi,

nematodes and viruses. A number of bacterial species have been recognized as suppress of plant pathogens, such as *Curtobacterium*, *Pseudomonas*, *Bacillus*, *Microbacterium*, *Kluyvera*, *Alcaligenes*, *Flavobacterium* and *Clavibacter* (Inderiati and Franco 2008; Nagendran et al 2013). In a study Joe et al (2012), reported *Achromobacter xylosoxidans* against *Magnaporthe oryzae* in rice. *Pseudomonas* sp., *Pantoea* sp. investigated against as biocontrol agents *Agrobacterium tumefaciens, Allorhizobium vitis* in the grapevine (Asghari et al 2019). *Bacillus subtilis* reported against *Rhizoctonia solani, Fusarium verticelloides, Sclerotium rolfsii* in rice (Yehia et al 2020). Abiotic stresses impact agricultural crop productivity and soil microbial activity. Microbes have a crucial part in agricultural plants' adaptive method and increasing tolerance to abiotic stresses.

Soil barring microbes belongs to diverse genera including *Azotobacter*, *Aeromonas, Azospirillum*, *Achromobacter*, *Bacillus*, *Enterobacter*, *Klebsiella, Pseudomonas* and *Variovorax* these microbes have been displayed to enhance plant growth under drought stress condition (Kumar et al 2019; Rai et al 2020). Water flooding microbes isolate from soil such as *Aquabacterium, Clostridium, Flavobacterium, Ilumatobacter, Bacillus, Solirubrobacter, Arenimonas* and *Mycobacterium* (Furtak et al 2020). The thermotolerant microbes, *Achromobacter spanius, Alcaligenes faecalis* and *Staphylococcus succinus* isolated from wheat rhizosphere (Verma et al 2019). Salt-tolerant plant growth-promoting microbes ACC deaminase-producing *Burkholderia pyrrocinia* strain isolated from the tea rhizosphere (Han et al 2021).

5. Technical programme

5.1 Experiment 1: Isolation and characterization of microbes associated with crops growing in hilly regions

5.1.1 Location: Eternal University Agricultural farm and microbial Biotechnology Laboratory, Department of Genetics, Plant Breeding and Biotechnology, Dr. Khem Singh Gill Akal Collage of Agriculture

5.1.2 Methodology: Survey and collection of samples from different cereal crops (Wheat, Maize, Barley and Oats) growing in Sirmaur district, Himachal Pradesh. Isolation of rhizospheric microbes using different growth media such as: Nutrient agar medium, King's B agar, T3 agar, Tryptic soy agar, Potato dextrose agar, Rose Bengal agar etc. Morphological and cultural characterization of isolated microbes on the basis of size (in mm), shape (spherical/rod-like/spiral/filamentous/irregular), color (pigmented/non-pigmented), margin (lobate/wavy/smooth), surface (smooth/rough/wrinkled) and polysaccharides producer.

5.1.3 Observation to be recorded: CFU count

5.1.4 Statistical analysis: Nil

5.2 Experiment 2: Screening of isolated microbes for mineral solubilizing attributes

5.2.1 Location: Microbial Biotechnology Laboratory, Department of Genetics, Plant Breeding and Biotechnology, Dr. Khem Singh Gill Akal Collage of Agriculture

5.2.2 Methodology: Screening of microbes for phosphorus, potassium, zinc and selenium solubilization. Screening for production of siderophores, HCN, ammonia, ACC and IAA. Biological nitrogen attributes using acetylene reduced assay. Screen of biomolecules for various enzyme producers

5.2.3 Observation to be recorded: Selection of microbes on the basis of halo-zone around the colony in case of solubilization of phosphorus, potassium, zinc and selenium. Observation of positive and negative results of bacteria for different PGP attributes

5.2.4 Statistical analysis: Nil

5.3 Experiment 3: Molecular characterization of selected mineral solubilizing microbes

5.3.1 Location: Molecular Biology Lab, Department of Genetics, Plant Breeding and Biotechnology, Dr. Khem Singh Gill Akal Collage of Agriculture

5.3.2 Methodology:

- Genomic DNA extraction from selected mineral solubilizing microbes
- Amplification of 16S rRNA gene
- Purification and sequencing for PCR product
- Sequences analysis using codon code aligner v.4.0.4 for Contig formation
- BLASTn analysis for identification of microbes with available database in NCBI, GeneBank
- A phylogenetic tree construction using MEGA 4.0.2 is know the taxonomical affiliates of microbes

5.3.3 Observation to be recorded: Observation of DNA and PCR product quantification in agarose gel

5.3.4 Statistical analysis: Nil

5.4 Experiment 4: Development of microbial consortium using of mineral solubilizing microbes

5.4.1 Location: Microbial Biotechnology Laboratory, Department of Genetics, Plant Breeding and Biotechnology, Dr. Khem Singh Gill Akal Collage of Agriculture

5.4.2 Methodology:

- Selection of potential mineral solubilizing microbes
- Compatibility test
- Growth kinetics of individual bacteria as well as in consortium

- Stability test of consortium up to six month

5.4.3 Observation to be recorded: Growth of single bacteria and microbial consortium. Observation of compatibility among bacteria and their survivability in liquid formation

5.4.4 Statistical analysis: Nil

5.5 Experiment 5: Evaluation and validation of mineral solubilizing microbes for plant growth promotion under controlled and natural conditions

5.5.1 Location: Greenhouse and Experimental farm, Machher

5.5.2 Methodology:

- Seed germination bioassays
- Evaluation of microbial consortium for plant growth on different cereal crops under both *in-vitro* (greenhouse conditions) and *in-vivo* conditions (Experimental farm, Machher)
  - Growth parameter (Plant height, fresh and dry weight of shoot and root)
  - Physiological parameters (chlorophyll, carotenoids, total soluble sugar, phenolic and flavonoid content) and nutrient uptake analysis using atomic absorption spectroscopy
  - Yields estimation (kg/ha) in field conditions

5.5.3 Observation to be recorded: Growth and seed germination in agar plate inoculated with bacteria. Growth and physiological parameter of crops inoculated with single and microbial consortium under the control and natural conditions.

5.5.4 Statistical analysis: t-test

6. Collaboration (if any): Nil

PLAN OF RESEARCH WORK

S.N.	Objectives	When to be attained					
		1st Year		2nd Year		3rd Year	
		I	II	I	II	I	II
1.	Isolation and characterization of microbes associated with crops growing in hilly regions						
2.	Screening of isolated microbes for mineral solubilizing attributes						
3.	Molecular characterization of selected mineral solubilizing microbes						
4.	Development of microbial consortium of mineral solubilizing microbes for plant growth and enhanced crop production						
5.	Evaluation and validation of mineral solubilizing microbes for plant growth promotion under controlled and natural conditions						***

*** Thesis writing

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Rubee Singh
(Rubee Devi)

Approved

Arumon 30/11/2021
(Dr. Archna Suman)

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Course of Study	Ph.D. Biotechnology
Name of Major Advisor	Dr. Ajar Nath Yadav
Department	Department of Genetics, Plant Breeding and Biote
Acceptable Maximum Limit	10%
Submitted By	euplagiarism@eternaluniversity.edu.in
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Rubee Singh
Signature of Student

Signature of Major Advisor

15/11/21
Head of the Department

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Report of the Research Degree Committee on Synopsis of Master's /Ph.D Thesis/Dissertation Research Problem

We have critically examined the synopsis of Thesis/Dissertation Problem entitled :**MINERAL SOLUBILIZING MICROBES ASSOCIATED WITH DIFFERENT CROPS AND THEIR FUNCTIONAL ROLES FOR PLANT GROWTH AND CROP PRODUCTIVITY** submitted by : **Ms. Rubee Devi** Registration No :**BS19PSBT002** for M.Sc/M. Tech/Ph.D degree in the subject of: **Biotechnology** to the Dr. KS Gill Akal College of Agriculture of the Eternal University, Baru Sahib(HP). It is certified that the research problem in original; programme of work is properly planned, feasible to carry out and is of good academic and practical significance.

Ms/Mr.: **Ms. Rubee Devi** gave a presentation on the above mentioned research problem to the Research Degree Committee and other faculty members and students on **November 24, 2021** and his/her performance is Satisfactory/~~Unsatisfactory.~~ Asuman

Synopsis is recommended for acceptance in by the Eternal University.

Asuman 30/11/2021
(Signature, Name and Address of of External Expert)

(Dr. Archna Suman)
Principal Scientist
Division of Microbiology
IARI, New Delhi-110012

1. _____ (Dr. B.S. Sohal)
(Chairperson, Research Degree Committee)

2. _____ Dr. Ajeet Nath Pradeep (Major. Ad
(Member, Research Degree Committee)

3. _____ Dr. Sunil Kumar
(Member, Research Degree Committee)

4. _____ Dr. Imran Shah
(Member Research Degree Committee)

5. _____ Dr. Vikrant Tyagi
(Member, Research Degree Committee)

6. _____ Dr. Deep Chandra
(Member, Research Degree Committee)

Recommendation

(Dean, Post-graduate Studies)

Approved/Not Approved

Vice Chancellor